JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 455–457 Vol. 43, No. 1
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.455–457.2005

High Prevalence of Respiratory Viral Infections in Patients Hospitalized in an Intensive Care Unit for Acute Respiratory Infections as Detected by Nucleic Acid-Based Assays

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Received 8 April 2004/Returned for modification 1 June 2004/Accepted 24 September 2004

Forty-seven bronchoalveolar lavages (BAL) were obtained from 41 patients with acute pneumonia attending an intensive care unit. By molecular diagnosis, 30% of total BAL and 63% of bacteria-negative BAL were positive for respiratory viruses. Molecular detection allows for high-rate detection of respiratory viral infections in adult patients suffering from severe pneumonia.

Severe community-acquired pneumonia is caused by bacterial infections in around 60% of cases (7, 20), and it requires admission to an intensive care unit (ICU) for about 10% of patients. Pneumonia is also the most commonly reported nosocomial infection in the ICU, with an incidence ranging from 7 to 40%, and it is often attributed to bacteria (9, 14). Respiratory viral infections constitute the second etiology of community-acquired pneumonia (5, 8, 11, 18) and have long been considered a simple differential diagnosis, without practical issues for the patient. However, the diagnosis of viral respiratory infection has recently appeared more and more relevant for medical care (1, 28). First, respiratory syncytial virus, influenza viruses, parainfluenza viruses, and adenoviruses have been identified as significant pathogens of community-acquired and nosocomial respiratory infections (16, 22, 26, 27). Second, new nucleic acid-based assays have been shown to be more sensitive than conventional techniques (13, 15, 25). Third, some respiratory viral infections, including influenza, enterovirus, and adenovirus, may now benefit from specific antiviral treatment (3, 4, 23). The aim of the present study was to evaluate the usefulness of molecular diagnosis of respiratory viral infections in a nonselected population of patients admitted to a medical ICU for severe pneumonia.

Forty-seven bronchoalveolar lavages (BAL) were prospectively obtained from 41 patients (14 females; mean age, 56 years) with community- or hospital-acquired pneumonia admitted to the medical ICU of Hôpital Européen Georges Pompidou, Paris, France, according to classical criteria (19). Two milliliters of total BAL was used for viral culture and immunofluorescence (IF) testing, whereas 1 ml was frozen at −80°C for subsequent molecular analysis. Clinical specimens underwent conventional viral culture in MRC5 and Vero cells, rapid culture in MRC5 cells, and IF by standard methods, as previously described (1, 21). Bacteria and fungi were isolated from BAL fluids, as previously described (10). Nucleic acids were extracted from 200 μl of total BAL using RNA and DNA extraction protocols on a silica column system. For RNA viruses, cDNA was produced by reverse transcription (RT) using random hexamers primers (Hexaplex; Prodesse Inc., Waukesha, Wis.). Parainfluenza virus types 1, 2, and 3, influenza virus types A and B, and respiratory syncytial virus types A and B were detected by a multiplex RT-PCR–enzyme hybridization assay (Prodesse Inc.) as described previously (13). Briefly, PCR amplification was performed by adding Super-Mix (Prodesse Inc.) and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer) to the newly synthesized cDNA. After denaturation at 95°C for 2 min, 40 PCR cycles were performed as follows: two cycles at 95°C for 1 min, 55°C for 45 s, and 72°C for 45 s, and then 38 cycles at 94°C for 1 min, 60°C for 45 s, and 72°C for 45 s, followed by an additional 7 min at 72°C to complete partial polymerizations. PCR products were then purified using a QIA Quick Purification kit (QIAGEN Inc.), denaturated, and added to a 96-well neutraviden-coated microtiter plate (Prodesse Inc.). Peroxidase-labeled probe solutions 1 to 7 (Prodesse Inc.), specific to each virus, were added each to a single well. A capture and hybridization reaction was then carried out for 1 h at 42°C, and substrate solution was added to each well. After 10 min, the reaction was stopped and the optical density of each well was measured at 450 nm on a spectrophotometer. The positive cutoff value was calculated as three times higher than the negative control, or >0.400. All samples tested by Hexaplex assay were further tested by confirmatory in-house RT-PCR using other virus target sequences, as previously described (6, 12). Human metapneumovirus, picornavirus, and coronavirus were detected by in-house RT-PCR, as previously described (2, 17, 24). Adenovirus DNA and herpes virus (HSV) DNA (HSV-1 and -2, varicella-zoster virus,

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Table 1. Conventional and molecular diagnosis of principal respiratory viral infection in the 47 BAL samples from 41 patients suffering from acute respiratory illness

Virus	Conventional diagnosis	Molecular diagnosis
Influenza virus A	1	7
Influenza virus B	0	1
Parainfluenza virus 1	0	1
Parainfluenza virus 2	0	1
Parainfluenza virus 3	0	0
Respiratory syncytial virus A	0	0
Respiratory syncytial virus B	0	2
Picornavirus	0	0
Human metapneumovirus	0	0
Human coronaviruses	0	0
Adenovirus	0	2
Total	1 (2.1%)	14 (29.8%)

cytomegalovirus [CMV], Epstein-Barr virus, and human herpesvirus 6) were detected by PCR (Adenovirus and Herpesconsensus; Argène-Biosoft, Varilhes, France).

Results are depicted in Table 1. By IF testing, 1 of 47 (2.1%) BAL was found positive for influenza A infection. By molecular diagnosis, 14 of 47 (29.8%) BAL were positive for respiratory viruses. The Hexaplex assay was positive in BAL from 11 patients, including eight samples positive for influenza virus (seven of type A and one of type B), two for respiratory syncytial virus type B, and two for parainfluenza virus (one of type 1 and one of type 2). One patient had two samples positive for influenza A virus in a 1-week interval. BAL samples from two other patients were positive for adenovirus. All positive as well as negative results obtained by the Hexaplex assay were confirmed by single-virus-specific in-house PCR. In total, 14 respiratory viruses were detected by molecular assays in 13 (31.7%) of 41 patients, while only one patient was determined to be infected by influenza virus by IF. Except influenza and parainfluenza viruses (which were unable to grow in MRC-5 and Vero cells), no viral culture was positive. According to the respiratory virus molecular detection results, etiologies of pneumonia are depicted in Fig. 1. The results show that among the BAL samples negative for bacteria at inclusion, 63% (12 out of 19) were actually positive for a respiratory virus. In three patients with bacteria-positive BAL, HSV-1 (2) and CMV (1) were isolated by culture, and in two patients infected by influenza A virus, HSV-1 was also isolated. Using Argene-Biosoft PCR, all positive and negative BAL samples were confirmed as positive or negative for herpesviruses, respectively.

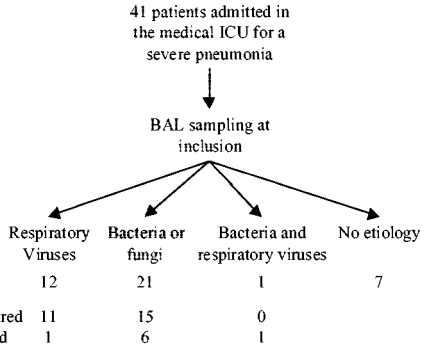

FIG. 1. Pneumonia etiology of patients admitted to the medical ICU. For the patients admitted to the medical ICU for a severe pneumonia, the number of respiratory viruses, bacteria, or fungi detections in the BAL fluid collected at inclusion as well as community or hospital acquisition of infection are indicated.

Five patients with virus-positive BAL died as a consequence of their pneumonia (influenza virus, 2; respiratory syncytial virus, 1; parainfluenza virus, 1; adenovirus, 1). All five suffered from an underlying disease (ataxia telangiectasia, silicosis, human immunodeficiency virus infection, renal transplantation and diabetes, and ischemic cardiopathy). All five patients had a bacterial superinfection and died from severe respiratory distress syndrome. Among patients infected by respiratory virus, one (8%) had an adenovirus infection following cardiac surgery, whereas all other respiratory virus infections were community acquired (Fig. 1). Mean age, mortality rate, cellular contents of BAL, number of days in ICU, and duration of invasive ventilation did not differ between patients with virus-infected BAL and those with bacteria-infected BAL (data not shown).

In the present series of severe pneumonia, viral infections were detected by using nucleic acid-based diagnosis procedures in BAL samples from nearly one-third of the patients; when only patients without bacterial etiology were considered, two-thirds of samples were found to be positive for respiratory virus. The molecular assays we used to detect respiratory viruses had a higher sensitivity than the conventional virological assays routinely used in our institution. These features are in keeping with previous reports (15, 25) showing that the search for respiratory viruses in BAL fluid by either viral culture or direct antigen testing dramatically underestimates the possibility of respiratory virus etiology, even in the case of acute respiratory infections not caused by bacterial infection. The multiplex RT-PCR associated with the enzyme hybridization assay allowed us to detect with high specificity the majority of respiratory viruses likely to be associated with acute respiratory syndromes, including influenza virus, respiratory syncytial virus, and parainfluenza virus. The five patients suffering from acute respiratory infections without bacterial etiology at admission and who died from their pneumonia were all found to be infected by a respiratory virus. In these patients, the viral pneumonia appeared to be community acquired, whereas nosocomial viral infection was observed in only one case. The respiratory viral infection likely constituted the primary cause of death and likely caused the worsening of the underlying morbid condition. These findings demonstrate that respiratory viruses detected by means of a molecular biology procedure may have marked clinical significance for determining causal agents in adult patients presenting with acute respiratory deficiency.

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